

1/11  
SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

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<212> DNA

<213> Escherichia coli K12

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accaactccc agccgtggca ttttattggt gccagcacgg aagaaggtaa agcgcgtggt 180  
gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgccctcgac 240  
gtcgtggtgt tctgtgcaaa aaccgcgatg gacgatgtct ggctgaagct ggttgttgac 300  
caggaagatg ccgatggccg ctttgccacg ccggaagcga aagccgcgaa cgataaaggt 360  
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aaaggctaca ccagtctggt ggttgttccg gtaggtcatc acagcgttga agattttaac 600  
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<212> DNA

2/11

&lt;213&gt; Pseudomonas putida JLR11

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agcgatcttc ctgtggatga gcagatgctg agctgggaga tcgaggcggc ccagtcagcc	180
tcgacttcct cgaacctgca agcttggagc gtgctcgccg tggggatcg cgagcgtctc	240
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gcaggtatcg actatctaga aagctacacc gtcgggtgtg tagatgcagc tctggccgct	420
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cgcaaccacc cggaagcgat gtccgaggag cttggcctgc caaacgacac ttctgctgta	540
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&lt;211&gt; 1066

&lt;212&gt; DNA

&lt;213&gt; Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (88)..(858)

&lt;223&gt; Coding sequence for nfnB gene

&lt;220&gt;

&lt;221&gt; misc\_feature

3/11

&lt;222&gt; (250)..(267)

&lt;223&gt; Cys tags

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (160)..(177)

&lt;223&gt; His tags

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (268)..(285)

&lt;223&gt; primer

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (996)..(1010)

&lt;223&gt; primer

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 Met Gly Ser Ser His His His His His  
 1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162  
 His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr  
 10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat 210  
 Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp  
 30 35 40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258  
 Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala  
 45 50 55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306  
 Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu  
 60 65 70

4/11

caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val 75 80 85	354
gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly 90 95 100 105	402
aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val 110 115 120	450
gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val 125 130 135	498
gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys 140 145 150	546
gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys 155 160 165	594
gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn 170 175 180 185	642
gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val 190 195 200	690
ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu 205 210 215	738
aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His 220 225 230	786
agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln 235 240 245	834
aac atc acc tta acc gaa gtg taa ttctctcttg ccgggcatct gcccggtat Asn Ile Thr Leu Thr Glu Val 250 255	888
ttcctctcag attctcctga tttgcataac cctgtttcag caagcttcgt catcataggc	948
tgctgttgaa gcttgcggcc gcactcgagc accaccacca ccaccactga gatccggctg	1008
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&lt;210&gt; 4

&lt;211&gt; 256

5/11

&lt;212&gt; PRT

&lt;213&gt; Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (250)..(267)

&lt;223&gt; Cys tags

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (160)..(177)

&lt;223&gt; His tags

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (268)..(285)

&lt;223&gt; primer

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (996)..(1010)

&lt;223&gt; primer

&lt;400&gt; 4

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Arg	Gly	Ser	His	Met	Ala	Ser	Met	Thr	Gly	Gly	Gln	Gln	Met	Gly	Arg
			20					25					30		

Gly	Ser	Cys	Cys	Cys	Cys	Cys	Cys	Asp	Ile	Ile	Ser	Val	Ala	Leu	Lys
		35					40					45			

Arg	His	Ser	Thr	Lys	Ala	Phe	Asp	Ala	Ser	Lys	Lys	Leu	Thr	Pro	Glu
	50					55					60				

Gln	Ala	Glu	Gln	Ile	Lys	Thr	Leu	Leu	Gln	Tyr	Ser	Pro	Ser	Ser	Thr
65					70					75					80

6/11

Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys  
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg  
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala  
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp  
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg  
145 150 155 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu  
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly  
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala  
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser  
210 215 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala  
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Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val  
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<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

<220>

7/11

&lt;221&gt; CDS

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&lt;221&gt; misc\_feature

&lt;222&gt; (190)..(225)

&lt;223&gt; primer

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (190)..(207)

&lt;223&gt; cys tag

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (936)..(956)

&lt;223&gt; primer

&lt;400&gt; 5

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Met Gly Ser Ser His His His His His  
1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162

His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr  
10 15 20 25

ggc gga cag caa atg ggc cgc gga tcc tgt tgc tgt tgc tgt tgc agc 210

Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser  
30 35 40

ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca 258

Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro  
45 50 55

gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat 306

Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His

8/11

60	65	70	
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agc tgg gcg atc gcg gcg gcc cag tca gcc tcg act tcc tcg aac ctg Ser Trp Ala Ile Ala Ala Ala Gln Ser Ala Ser Thr Ser Ser Asn Leu 90 95 100 105			402
caa gct tgg agc gtg ctc gcc gtg cgg gat cgc gag cgt ctc gcg agg Gln Ala Trp Ser Val Leu Ala Val Arg Asp Arg Glu Arg Leu Ala Arg 110 115 120			450
ctt gcc cga ctg tcc ggt aac cag cgc cat gtc gag cag gca ccg ctg Leu Ala Arg Leu Ser Gly Asn Gln Arg His Val Glu Gln Ala Pro Leu 125 130 135			498
ttc ctg gtc tgg ctc gtg gac tgg tca cgc cta cgc cga cta gcc aga Phe Leu Val Trp Leu Val Asp Trp Ser Arg Leu Arg Arg Leu Ala Arg 140 145 150			546
acc ctt cag gca ccg act gca ggt atc gac tat tta gaa agc tac acc Thr Leu Gln Ala Pro Thr Ala Gly Ile Asp Tyr Leu Glu Ser Tyr Thr 155 160 165			594
gtc ggt gtt gta gat gca gct ctg gcc gct cag aac gcc gca cta gct Val Gly Val Val Asp Ala Ala Leu Ala Ala Gln Asn Ala Ala Leu Ala 170 175 180 185			642
ttc gag gcc caa gga ctg gga atc gtt tac atc ggc gga atg cgc aac Phe Glu Ala Gln Gly Leu Gly Ile Val Tyr Ile Gly Gly Met Arg Asn 190 195 200			690
cac ccg gaa gcg atg tcc gag gag ctt ggc ctg cca aac gac act ttc His Pro Glu Ala Met Ser Glu Glu Leu Gly Leu Pro Asn Asp Thr Phe 205 210 215			738
gct gta ttt ggc atg tgc gtc ggt cat ccc gat ccg gca cag ccc gcc Ala Val Phe Gly Met Cys Val Gly His Pro Asp Pro Ala Gln Pro Ala 220 225 230			786
gag atc aag cca cgc ctg gcg caa tca gtg gtg ctt cac cgt gag cgc Glu Ile Lys Pro Arg Leu Ala Gln Ser Val Val Leu His Arg Glu Arg 235 240 245			834
tat gag gcc acc gag gca gag gcg gtt tca gtt gct gcc tat gac cga Tyr Glu Ala Thr Glu Ala Glu Ala Val Ser Val Ala Ala Tyr Asp Arg 250 255 260 265			882
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tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly 285 290 295			978
aga cac cgc ttg cga gat gca tta aac acc cta ggt ttc ggc ctg cgc			1026



9/11

Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg  
300 305 310

tga gatagtgaga tatcccatgc ctattcccgc cgccctgaac cggagcacta 1079  
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<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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35 40 45

10/11

Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp  
50 55 60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser  
65 70 75 80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala Ala  
85 90 95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala  
100 105 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn  
115 120 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp  
130 135 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala  
145 150 155 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala  
165 170 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly  
180 185 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu  
195 200 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val  
210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala  
225 230 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu  
245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His  
260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg  
275 280 285

11/11

Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala  
290 295 300

Leu Asn Thr Leu Gly Phe Gly Leu Arg  
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<212> DNA

<213> Escherichia coli

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ggatccgata tcatttctgt cgcc

24

<210> 8

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<212> DNA

<213> Escherichia coli

<400> 8

cgatcatcata ggctgctggt gaagctt

27

<210> 9

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

<400> 9

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42